STUDIES ON THE CHEMICAL NEEDS OF AMŒBA PROTEUS: A CULTURE METHOD

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Introduction

The chemical needs of plants have been fairly well worked out by the culture method, but knowledge regarding the needs of animals is very inadequate. This is largely due to the fact that plants can be grown in synthetic solutions containing only inorganic salts, while animals require in addition some organic material. For example, *Amœba proteus* feeds on *Chilomonas paramecium; Chilomonas* in turn requires some organic nutrient.

In the culture of protozoa, the organic nutrient has usually been added in the form of timothy hay or grain. These substances, however, contain a considerable amount of physiologically active salts, which diffuse out into the culture and alter it in an unknown way. Under these conditions, since the kind and concentration of chemical elements are not known and do not remain constant, it is difficult to ascertain the kind and number and relative amount of elements necessary for the maintenance and growth of animal protoplasm. By a method to be described below, variation in the salt composition of the medium is fairly accurately controlled, thereby making it possible to ascertain the relative importance of individual elements in rhizopod protoplasm.

Methods and Results

Amœba proteus (Leidy) was used in all the observations made. It was derived from stock cultures made by adding a grain of rye to a mixture of half-spring-half-distilled water in finger bowls with subsequent inoculation with amœbæ and *Chilomonas*.

Two series of experiments were performed in which variation in the salt composition of the medium was controlled. These experiments were made as follows: Five balanced salt solutions[2] were prepared as

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[2] Kahlbaum (analysis grade) chemicals (except Merck's blue label $Ca_3(PO_4)_2$) and water redistilled in pyrex glass were used in all solutions. According to recent investigations by Williams and Jacobs (1931), certain brands of C. P. sodium chloride contain a toxic impurity whose destructive effect overbalances any beneficial effect the sodium chloride itself may have. It should be noted that the Kahlbaum salt, which Williams and Jacobs found to be the least toxic of the five brands tested, was used in these experiments.

indicated in Table I. The first, Chalkley solution,[3] contained nine chemical elements in the form of salts, the rest contained fewer. Then 20 cc. of the solution under consideration was put into each of five 50 cc. pyrex glass beakers. Numerous amœbæ were now removed from a stock culture and put into a pyrex glass beaker containing 25 cc. redistilled water and left a few minutes, after which all that were in good condition were transferred to another pyrex glass beaker containing redistilled water. This was repeated a third time. Then 10 ± amœbæ, washed free of culture fluid, were put into each beaker containing the different salt solutions. Specimens of *Chilomonas* were now concentrated by means of a centrifuge; they were then added to a large quantity of redistilled water and again concentrated, after which 0.2 cc. of the resulting dense culture of *Chilomonas* was added to each beaker. This organism served as food for the amœbæ.

During the process of washing in redistilled water, practically all of

TABLE I

Chemical Composition of the Solutions Tested as Culture Media for Amœba proteus

Compound	(1)	(2)	(3)	(4)	(5)
	gram	*gram*	*gram*	*gram*	*gram*
$NaCl$	0.08	0.08	0.08		
$NaHCO_3$	0.004	0.004	0.004		
KCl	0.004	0.004		0.004	0.004
$CaCl_2$	0.004	0.004	0.004	0.004	0.004
$CaH_4(PO_4)_2$	0.002	0.002	0.002	0.002	0.002
$Mg_3(PO_4)_2$	0.002		0.002	0.002	0.002
$Ca_3(PO_4)_2$					0.002
H_2O (cc.)	1000	1000	1000	1000	1000

the original culture fluid with its unknown chemical content was eliminated from both amœbæ and *Chilomonas*. No nutrient in the form of hay or grain was added to the salt solutions in the beakers. Therefore, since the composition of *Chilomonas* was the only unknown chemical factor in these solutions and since this factor was the same in all, any variation in the vital processes in *Amœba* must be due to known differences in the chemical constitution of the solutions.

In both series of experiments, all of the beakers were kept in diffuse light. Each was covered with a glass plate to reduce evaporation. The temperature during the course of the tests was fairly constant and was the same for all. Observations with reference to the number of amœbæ and their physiological condition were made with a binocular microscope. The condition of *Chilomonas* was also noted. These

[3] This solution is a modification by Chalkley of one used by Drew in tissue culture work; it has proven a reliable culture medium for *Amœba proteus*.

observations were made every day or every other day during the first half of the experiments; a final observation was made several days later, at which time the experiments were discontinued. The amœbæ became quite numerous in some of the solutions and the process of counting them became increasingly difficult. The latter was facilitated by marking quadrants on the bottom of the beakers with a China marking pencil and then counting the amœbæ in each section separately.

In the first experiment three salt solutions were tested as follows: (1) Chalkley solution, (2) Chalkley solution without $Mg_3(PO_4)_2$ (Table I), and a mixture of half Sieur de Mons spring water with half redistilled water.[4] The experiment continued 21 days, but at the end

TABLE II

Effect of Different Salt Solutions on Growth in Amœba proteus

Solution	Days after inoculation					Average progeny per original individual
	0	4	10	17	21	
	Number of animals present					
(1) Chalkley solution	41	72	214	446	696	16.9
(2) Same less $Mg_3(PO_4)_2$	42	72	179	249	263	6.26
Mixture of half-spring-half-redistilled water	45	75	147	267	310	6.8

of a week, specimens of *Chilomonas* were not abundant in the solutions; consequently, 0.2 cc. of a fresh, washed, and concentrated culture was added to each beaker.

The hydrogen-ion concentration of all the cultures remained between pH 6.3 and 6.6 during the experiment. The results obtained regarding the number of amœbæ present are given in Table II. Each number in the first five columns of this table is the total number of amœbæ in the five beakers of the stated solution at the stated time. Each number in the last column is the average progeny produced from each original amœba by successive fissions during the course of the experiment; it is obtained by dividing the number in column five by the corresponding number in column one.

This table shows that there was growth in each of the three solutions, as indicated by increase in the number of individuals, and that

[4] Ordinary spring water diluted with distilled water is usually a favorable medium for culturing amœbæ. In this experiment water from the Sieur de Mons spring, Mt. Desert Island, was used as the control solution. This spring water is a very weak solution of various salts ordinarily found in soil. Chalkley solution, however, was found to be a more favorable medium.

Amœba proteus can live and reproduce for more than 21 days in a synthetic salt solution with *Chilomonas paramecium* the only organic material present. It indicates that Chalkley solution is the most favorable, and Chalkley solution without $Mg_3(PO_4)_2$ is the least favorable of the three tested. The results obtained with spring water are of interest only in comparison and need not be considered further.

In the second experiment four solutions were tested in the same way as those in the preceding experiment. These salt solutions are described in Table I; *i.e.*, (1) Chalkley solution, (3) Chalkley solution without potassium salt, (4) Chalkley solution without sodium salts, and (5) Chalkley solution with the sodium salts replaced by calcium tribasic phosphate. The hydrogen-ion concentration of (1) and (3) remained between pH 6.4 and 6.6 and that of (4) and (5) between pH 6.2 and 6.4 during the 17 days of the experiment. The omission of sodium bicarbonate in (4) and (5) is responsible for this difference in hydrogen-ion concentration.

TABLE III

Effect of Different Salt Solutions on Growth in Amœba proteus

Solution	Days after inoculation					Average progeny per original individual
	0	2	4	6	17	
	Number of animals present					
(1) Chalkley solution	50	97	225	563	1956	39.1
(3) Same less potassium salt	50	85	210	565	1686	33.7
(4) Same less sodium salts	50	80	210	577	2404	48.1
(5) Same with sodium salts replaced by $Ca_3(PO_4)_2$	50	94	233	656	2676	53.5

The results obtained regarding the number of amœbæ produced are presented in Table III. The numbers have the same significance as in the preceding table. By referring to this table, it will be seen that there was little variation in the number of amœbæ present in the different solutions during the first part of the experiment but that there was considerable variation during the last part.

This variation on casual observation may not appear great, but when subjected to statistical analysis, it shows certain relations of some significance. Since the data were obtained on cultures (*i.e.*, groups of ten amœbæ) instead of on individuals, the mean progeny in five cultures of a solution was chosen as the relative value for that solution. The means with their probable errors and the difference between the means with their probable errors, computed from the same data as Table III, are given in Table IV.

It is considered reasonably certain that the mean of several measurements falls within three times its probable error; *i.e.*, the chances are 22.5 to 1 that it does. Table IV shows that the means of cultures (1) and (3) and also those of (4) and (5) are separated by about twice the sum of their probable errors whereas those of (1) and (4) are separated by more than four times the sum of their probable errors. Computation of the difference between the means shows the same relations; the difference between the means of (1) and (3) is 2.73 times the probable error, that between (4) and (5) 1.73 times, and that between (1) and (4) 5.25 times. It will be noted also that when the other solutions are compared with Chalkley solution, each of the differences between the means is probably significant, *i.e.*, in comparing (3) with (1), it is 2.73 times the probable error, (4) with (1) 5.25 times, and (5) with (1) 4.19 times. This indicates that the difference between (1) and (3) and also between (4) and (5) may or may not be

TABLE IV

Means and difference between the means of number of amœbæ present in the different solutions. Solutions arranged in ascending order of means. Based on same data as Table III.

Solution	Mean amœbæ per culture with p.e.	Difference between means with p.e.	$\frac{M_1-M_2}{p.e.}$
(3) Chalkley less K-salt	337.2±15.55		
		54.0±19.76	2.73
(1) Chalkley	391.2±12.26		
		89.6±17.06	5.25
(4) Chalkley less Na-salts	480.8± 7.03		
		54.4±31.47	1.73
(5) Chalkley less Na-salts plus Ca-salt	535.2±30.70	144.0±34.33	4.19

significant of an actual difference between the sample distribution in these solutions but that the differences between (1) and (4) and also between (1) and (5) are significant of actual differences and cannot be due to random sampling alone. Comparison of the distribution in the different solutions by the χ^2 method (*see* Pearson, 1914) indicates also that for any two solutions the variation in the number of amœbæ present cannot be due to random sampling alone. Consequently, since the cultures were set up with the same care, received the same treatment, and differed only in the salt content of the solutions, the observed variation in fission rate must be due, at least in part, to difference in the chemical composition of the solutions.

The data presented in Table III indicate therefore that Chalkley solution without sodium is more favorable than that with sodium, and

that Chalkley solution with the sodium salts replaced by calcium tribasic phosphate is still more favorable. It indicates also that the absence of potassium is detrimental because the amœbæ in the solution without it had the lowest fission rate of all. The difference in hydrogen-ion concentration of (4) and (5) as compared with (1) and (3) may be a factor in the more rapid fission rate in the former solutions, although the optimum is generally considered to be at pH 6.6–6.7.

In all solutions the amœbæ remained in good condition during the experiments; at each observation practically all were attached and moving in monopodal or bipodal form. Little change was observed in *Chilomonas* during the first week, and they were still in fair condition at the close of the experiments, although less rounded and plump than at the beginning. Detailed studies on the structural and physiological changes in *Chilomonas* during starvation are in progress.

Summary

1. *Amœba proteus* grows and reproduces for several weeks in a balanced salt solution containing potassium chloride, calcium chloride, calcium phosphate, magnesium tribasic phosphate, and *Chilomonas paramecium*.

2. They also grow in other solutions, but not so well; *e.g.*, in a solution containing sodium chloride and sodium bicarbonate in addition to the above salts.

3. The results obtained indicate that the presence of sodium is not only unnecessary but actually detrimental while that of magnesium and potassium is favorable if not essential for growth and reproduction in *Amœba proteus*.

4. By observing the effect on fission rate and other physiological processes of omitting various elements or altering their concentration in the solution, it is possible to ascertain the relative importance of the elements to the rhizopod protoplasm and the number and kind and amount necessary for growth.

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